Chapman, E. M., and Maloof, F. (1955). *Medicine (Baltimore)*, **34**, 261.
Curran, R., Eckert, H., and Wilson, G. M. (1958). *J. Path. Bact.*, **76**, 541.
Doniach, I. (1958). *Brit. med. Bull.*, **14**, 181.
Eckert, H., Green, M., Kilpatrick, R., and Wilson, G. M. (1960). *Clin. Sci.*, **20**, 87.
Goudie, R. B., Anderson, J. R., and Gray, K. G. (1959). *J. Path. Bact.*, **77**, 389.
Green, M., Fisher, M., Miller, H., and Wilson, G. M. (1961). *Brit. med. J.*, **2**, 210.
Greene, R. (1950). *J. Endocr.*, **7**, 1.
Irvine, W. J., Macgregor, A. G., and Stuart, A. E. (1962). *Lancet*, **2**, 843.
Jones, K. H., and Fourman, P. (1963). Ibid., **2**, 121.
Macgregor, A. G. (1963). *The Thyroid and its Diseases*, p. 19, edited by A. S. Mason. Pitman, London.
Riddell, V. (1962). *Brit. J. Surg.*, **49**, 465.
Roitt, I. M., and Doniach, D. (1958). *Lancet*, **2**, 1027.
Rose, N. (1963). Ibid., **2**, 116.
Schade, R. O. K., Owen, S. G., Smart, G. A., and Hall, R. (1960). *J. clin. Path.*, **13**, 499.
Segal, R. L., Silver, S., Yohalem, S. B., and Feitelberg, S. (1961). *Amer. J. Med.*, **31**, 354.
Sheline, G. E., and Miller, E. R. (1959). *Arch. intern. Med.*, **103**, 924.
Thorén, A., and Wijnbladh, H. (1956). *Acta endocr. (Kbh.)*, **22**, 224.
Volpe, R., Schatz, D. L., Scott, A., Peller, J. A., Vale, J. M., Ezrin, C., and Johnston, M. W. (1960). *Canad. med. Ass. J.*, **83**, 1407.
Wayne, E. J. (1954). *Brit. med. J.*, **1**, 411.
Werner, S. C., Coelho, B., and Quimby, E. H. (1957). *Bull. N.Y. Acad. Med.*, **33**, 783.
Whitesell, F. B., and Black, B. M. (1949). *J. clin. Endocr.*, **9**, 1202.
Williams, E. D., and Doniach, I. (1962). *J. Path. Bact.*, **83**, 255.

Chromosome Studies During Early and Terminal Chronic Myeloid Leukaemia

N. H. KEMP,* M.B., B.S.; J. L. STAFFORD, M.B., CH.B.; ROSEMARY TANNER, B.SC.

Brit. med. J., 1964, **1**, 1010–1014

The presence of the Philadelphia chromosome (Ph^1) is invariably associated with chronic myeloid leukaemia (Baikie *et al.*, 1960; Nowell and Hungerford, 1960a, 1960b, 1961; Tough *et al.*, 1961, 1962, 1963; Sandberg *et al.*, 1962b; Fitzgerald *et al.*, 1963), although its absence is probably not sufficient evidence to deny this diagnosis (Tough *et al.*, 1963). As all the patients so far reported had haematologically and, usually, clinically apparent leukaemia when the chromosome studies were first performed, the timing of the appearance of Ph^1+ cells, in relation to the initiation of the disease process, remains undefined.

The purpose of this communication is to demonstrate that Ph^1+ cells are present before the disease is even haematologically obvious and to stress the value of early definitive diagnosis whereby treatment might be started before conventional haematological criteria are available. We report the findings from one patient in whom Ph^1+ cells were observed before there was any haematological or clinical evidence of the leukaemia but who subsequently developed chronic myeloid leukaemia which rapidly progressed into an acute phase: a terminal myeloblastic proliferation was associated with further chromosome abnormalities. Also reported are the findings from four patients who presented as problems of diagnosis and in whom Ph^1+ cells were observed before the disease had become fully manifest. None of these cases had received antileukaemic treatment when they were first studied. Case 1 was studied during an investigation of the chromosome complement of bone-marrow cells from patients with polycythaemia vera, and the initial findings have been reported elsewhere (Kemp *et al.*, 1961).

Methods

Although examination of bone-marrow specimens by a "direct" method is preferable for the cytogenetic study of primary bone-marrow disease, it is not always practicable, especially with serial studies, for, as in Case 1, repeated marrow aspiration is not well tolerated by certain patients. So long as the leukaemic cells are circulating, and if every attempt is made to harvest them when they are dividing, peripheral blood specimens have, in our laboratory, proved more informative than has been the experience of certain other workers (Sandberg *et al.*, 1962a). The peripheral blood leucocytes were cultured by a modification of the technique of Moorhead *et al.* (1960), aliquots being harvested at regular intervals from the time when each specimen was first set up in order to make available for study any cells that divided during the early periods of culture but not subsequently; these would be missed if the cultures were not terminated until the conventional 48 hours or more had elapsed. When possible, leucocyte cultures with and without phytohaemagglutinin were studied in parallel. Exclusion of phytohaemagglutinin from the cultures prevents contamination with metaphases from transformed lymphocytes and permits any divisions that are seen to be attributed more confidently to an abnormal cell population present in the inoculum. The leucocytes from several specimens were set up in fresh homologous plasma as well as in autologous plasma, the former on occasion providing a more active culture.

Both short-term cultures (Ford *et al.*, 1958) and a "direct" method (Tjio and Whang, 1962) have been used for the study of bone-marrow specimens, the final preparations of either blood or marrow being made by air-drying. As is the practice with other workers, we have assessed the presence or absence of the Ph^1 chromosome only in cells in which all the small acrocentric chromosomes have been clearly defined.

Histochemical assessment of the polymorphonuclear neutrophil leucocyte alkaline phosphatase (L.A.P.) content was performed on peripheral blood specimens, using the modified azo-dye coupling technique and scoring method described by Hayhoe and Quaglino (1958).

* From the Haematology Department, St. George's Hospital, London.

Case 1

The patient was a man aged 39 in 1959. Polycythaemia rubra vera was first diagnosed in March 1959, when albuminuria, noted when he was undergoing treatment for chronic eczema, was being investigated. The findings at this time were: haemoglobin (Hb) 19.4 g./100 ml.; packed-cell volume (P.C.V.) 59%; white-cell count (W.B.C.) 25,200/c.mm. (neutrophils 68%, lymphocytes 25%, monocytes 7%); platelets 486,000/c.mm.

The patient was effectively treated, initially by venesection; later, in addition, he received three injections each of 5 mc. of ^{32}P. The haematological findings in this patient from the time of diagnosis until death are shown in Fig. 1.

In May 1959, during an attack of right lobar pneumonia, the W.B.C. rose to 50,000/c.mm., which included occasional myelocytes and metamyelocytes. However, when the infection was successfully treated the total leucocyte count returned to its previous level of approximately 20,000/c.mm., and immature granulocytes disappeared from the blood. During the course of the polycythaemia, and particularly after ^{32}P treatment, the patient suffered from attacks of gout; these were treated with probenecid, colchicine, and phenylbutazone—a total of 5.4 g. of the latter being given for this purpose between February 1960 and November 1961.

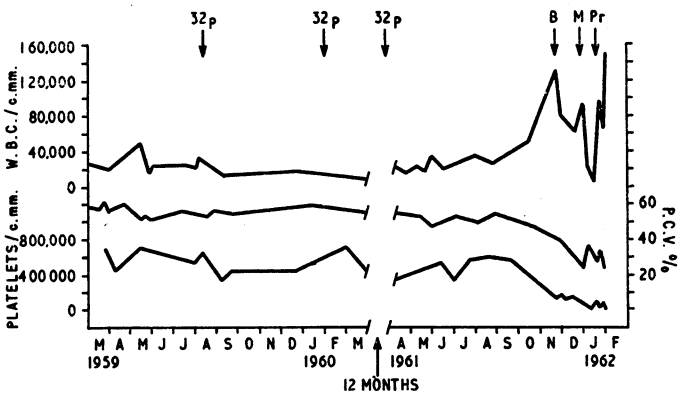

FIG. 1.—Case 1. Showing changes of packed cell volume, white blood cells, and platelet count from time of diagnosis. B=Busulphan. M=Mercaptopurine. Pr=Prednisone.

Chromosome studies were first performed on a specimen of bone-marrow taken in April 1961, at which time the peripheral blood picture was unexceptional for polycythaemia rubra vera. The results of this and subsequent chromosome investigations, together with the relevant haematological findings, are recorded in Table I.

The patient's progress was followed at frequent intervals, but there was little change until September 1961, when the W.B.C. rose rapidly and was accompanied by a fall in the Hb, P.C.V., and platelet count (Fig. 1). Neutrophil metamyelocytes, myelocytes, and eventually promyelocytes and occasional myeloblasts appeared in the blood, the W.B.C. reaching 132,000/c.mm. The picture was that of chronic myeloid leukaemia, and treatment with busulphan (6 mg. daily) was started on 20 November; this reduced the number of white cells circulating, including granulocyte precursors, but had little effect on the promyelocytes and blast cells. By the end of December 1961 the clinical and haematological picture had changed to that of a myeloblastic phase of chronic myeloid leukaemia: mercaptopurine (100 mg. daily) was given from 22 December in addition to the busulphan. By this treatment the W.B.C. was reduced to 3,800/c.mm. but the circulation of blast cells persisted.

When, because of severe thrombocytopenia, treatment had to be abandoned on 4 January 1962 after a total of 192 mg. of busulphan and 1.3 g. of mercaptopurine had been given, the W.B.C. rose rapidly to 153,000/c.mm. with myeloblasts and promyelocytes as the predominant cells. Despite prednisone (10 mg. t.d.s.) the patient died three weeks later with severe haemorrhagic manifestations of the then persistent thrombocytopenia. This was two-and-three-quarter years after the polycythaemia was initially diagnosed and four months after the leukaemic process first became evident in the peripheral blood.

Chromosome Analysis

The chromosome numbers of cells from a series of specimens are presented, with the relevant haematological data, in Table I, together with the incidence of the Philadelphia chromosome in the various cell lines.

The first specimen of bone-marrow (A), taken during the study of a series of patients with polycythaemia rubra vera (Kemp *et al.*, 1961), contained cells with the normal number of 46 chromosomes. However, in all the cells suitable for assessment, with an otherwise normal male karyotype, a chromosome indistinguishable from the Ph^1 was noted (Fig. 2). The possibility that chronic myeloid leukaemia might be developing in this patient was supported by the low neutrophil L.A.P. score of 6, although at this time the other haematological values were unexceptional for polycythaemia rubra vera.

TABLE I.—*Case 1. Chromosome Counts and Relevant Haematological Data Together with the Incidence of the Philadelphia Chromosome in the Various Cell Lines. All the Peripheral Blood Specimens were Cultured with Phytohaemagglutinin*

Specimen	Date	Total No. of Cells	Chromosome Number										Ph^1 %	Duration *in vitro* of Cultures Assessed (Hours)	W.B.C. c.mm.	N. %	L. %	M. %	E. %	B. %	Mm. %	My. %	Pro. %	Bl. %	L.A.P. Score	Treatment
			<44	44	45	46	47	48	49	50	51	<51	4N													
A marrow (culture)	21/4/61	10	1			9 (8/8)								100	6	16,500	76	18	4	1	1					6	Nil
B blood	5/5/61	17	1			16								0	66	22,500	64	25	5	3	1		2			1	"
C "	16/5/61	44	3		3	37 (8/31)	1							26	42, 48, 66, 72	18,800	81	10	4	2	2		1				"
D "	19/9/61	50	18		2	27 (20/24)	2 (1/1)					1 (1/1)		84	90, 96	36,500	60	21	6		1	8	4				"
E "	20/11/61	50	10	1	2	35 (33/33)	2 (2/2)							100	42	132,500	28	2				10	39	18	3		"
F "	30/11/61	48	3			40 (30/31)	5 (5/5)							97	24, 42	80,000	32	7		1	1	14	8	21	16		Busulphan
G "	27/12/61	4				2 (1/1)	2 (1/1)							100	48, 66	92,400	14	6				14	4	23	39		Busulphan mercaptopurine
H "	8/1/62	61	4		2	15 (2/9)	6 (5/6)	7 (7/7)	9 (9/9)		2 (2/2)		16 (16/16)	81	42, 48, 66	7,200	39	28	1			7	2	18	5		Nil
I "	15/1/62	59	2		1	21 (8/13)	1 (1/1)	4 (4/4)	12 (12/12)	10 (10/10)	2 (2/2)	1 (1/1)	5 (5/5)	89	18, 24, 42	3,800	44	40	2			2	4	4	4		"
J "	22/1/62	78	1		1	1 (1/1)	3 (3/3)	7 (7/7)	52 (52/52)	11 (11/11)	1 (1/1)	1 (1/1)		100	24	95,900	1	3	1			6	11	39	39		"

N.=Neutrophils. L.=Lymphocytes. M.=Monocytes. E.=Eosinophils. B.=Basophils. Mm.=Metamyelocytes. My.=Myelocytes. Pro.=Promyelocytes Bl.=Blast cells. Figures in parentheses=Incidence of the Ph^1 chromosome.

All the cells of the blood specimen B, taken two weeks after the bone-marrow, were Ph^1-, and it was only when myelocytes began to circulate that Ph^1+ cells were detected in the blood (C and D). The percentage of Ph^1+ cells increased as more immature granulocytes circulated until, in November 1961, when the blood was typical of chronic myeloid leukaemia, all the assessable cells contained the Ph^1 chromosomes (E). In subsequent specimens all the cells with 46 or more chromosomes were Ph^1+ except in cultures H and I, when some Ph^1- cells were observed. As these cells with a normal male karyotype were mostly present in the cultures that were harvested after 48 hours, they were considered to reflect the increased proportion of lymphoid cells present in these blood specimens at this time of relatively effective therapy due to mercaptopurine.

Initially the modal chromosome number of the marrow and blood specimens was 46. However, when a significant number of blast cells started to circulate, the proportion of hyperdiploid cells increased. In culture F four of the five cells with 47 chromosomes had the same karyotype containing an extra chromosome No. 21 or 22 (Denver). Although very few satisfactory metaphases were obtained from culture G, those with 46 and 47 chromosomes were present in equal proportions.

Cultures H, I, and J, taken after busulphan and mercaptopurine treatment had been stopped, contained many hyperdiploid cells. Cells with 49 and 50 chromosomes were prominent in I, while those with 49 chromosomes dominated culture J, which was established at the height of the terminal myeloblastic crisis. All the polyploid cells in H and I were Ph^1+ and were approximately tetraploid.

Karyotypes of 79 of the hyperdiploid cells from the last three cultures showed that there was usually more than one cell line within the various modes, though in the last culture, J, most of the cells with 49 chromosomes had the same karyotype (Fig. 3). In all of the hyperdiploid cells the extra chromosomes were indistinguishable from the normal set except that many cells contained an additional small acrocentric identical with the Ph^1. There were then two Ph^1s in most of the cells with 48 chromosomes and in all with more than 48. At least six chromosomes indistinguishable from a No. 3, a 4 or 5, a 6–12, a 13–15, a 19–20, and a Ph^1 were involved in the formation of the various interrelated cell lines. A simple system of successive non-dysjunction is easily invoked, which would account for the presence of all the cell lines observed by a stepwise evolution from an original 46 Ph^1+ cell.

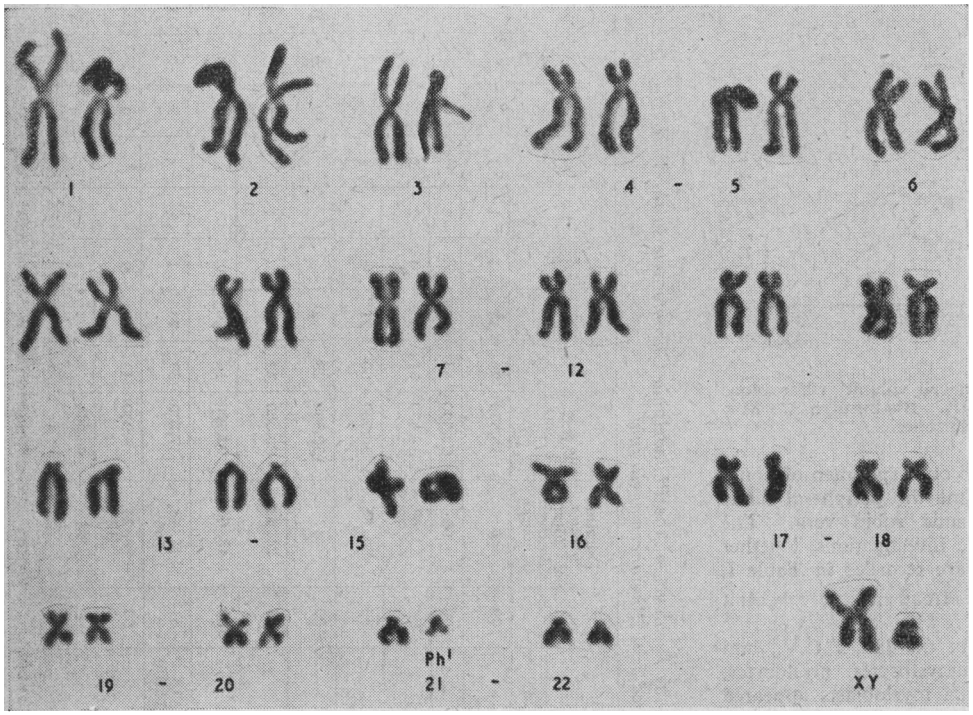

FIG. 2.—Case 1. Karyotype of 46 Ph^1+ cell from bone-marrow culture (A).

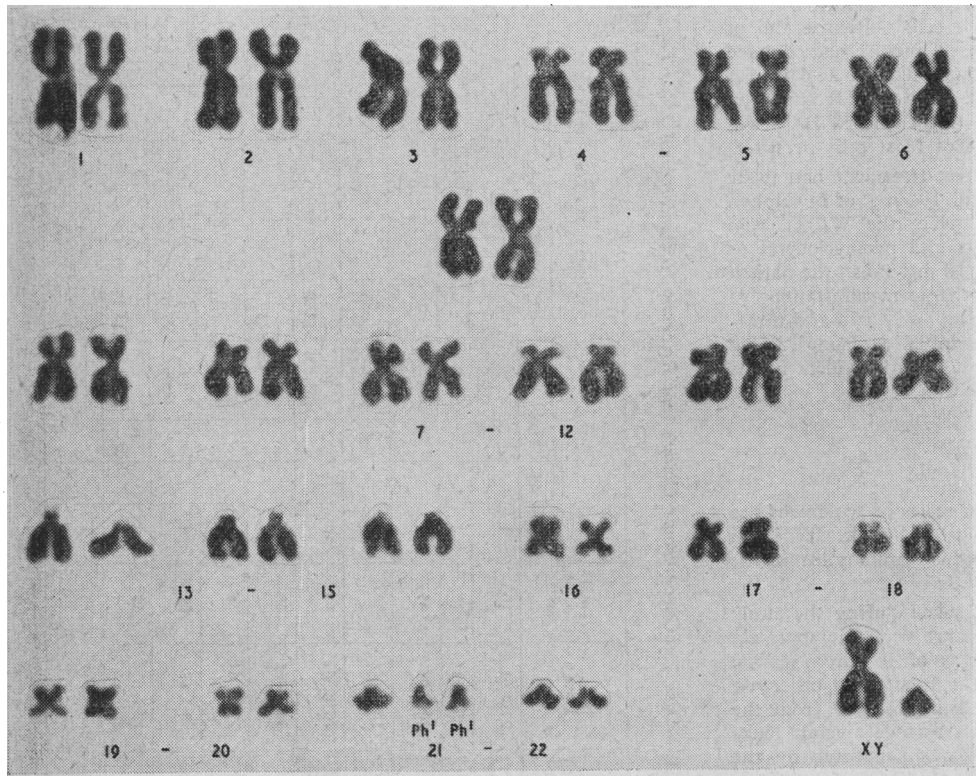

FIG. 3.—Case 1. Karyotype of cell line with 49 chromosomes from peripheral blood culture (J) showing two Ph^1 chromosomes and two extra large chromosomes. The relatively poor chromosome morphology was characteristic of the aneuploid cells in cultures H, I, and J.

Case 2

In September 1962 a raised W.B.C. was noted when the patient, a woman aged 24, was being followed-up after the surgical removal, in 1960, of a small fibroma of doubtful aetiology from her right forearm. She was well and there were no abnormal clinical findings; the values were Hb 12.4 g./100 ml.; W.B.C. 24,000/c.mm. (neutrophils 60%, lymphocytes 25%, monocytes 7%, eosinophils 8%). The leucocytosis persisted, and one month later, when the marrow showed increased granulopoietic activity, a few myelocytes and metamyelocytes were circulating.

Chromosome studies were performed in November 1962, when the W.B.C. was 48,000/c.mm. The findings are recorded in Table II. The W.B.C. continued to rise and was associated with a slight anaemia. In April 1963 the blood picture was Hb 10.4 g./100 ml.; W.B.C. 95,000/c.mm. (neutrophils 56%, lymphocytes 3%, monocytes

2%, eosinophils 8%, neutrophil myelocytes 17%, metamyelocytes 10%, promyelocytes 3%, blasts 1%); 1 nucleated red cell/100 white cells. The patient, apart from slight tiredness, remains well and has not so far received antileukaemic treatment. There are still no abnormal clinical findings and the spleen has never been palpably enlarged.

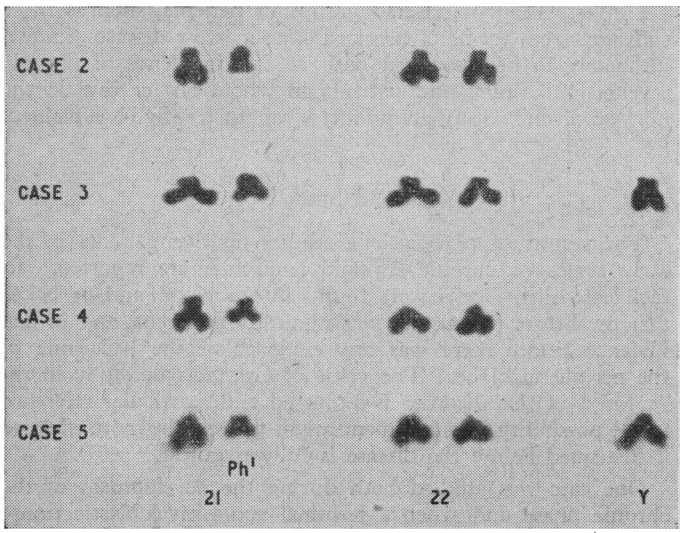

FIG. 4.—Cases 2–5. Chromosomes 21, 22, and Y showing the Ph¹ chromosome.

Case 3

A male Ghanaian aged 41 in 1962 attended hospital in October 1961 for investigation of a periostitis of the left tibia, having had pain and some swelling of the ankle over the previous 10 years. A raised W.B.C. was recorded early in 1962, when the findings were Hb 12 g./100 ml; W.B.C. 26,400/c.mm. (neutrophils 51%, lymphocytes 8%, monocytes 2%, metamyelocytes 19%, myelocytes 19%, blasts 1%); 2 nucleated red cells/100 white cells. The bone-marrow at this time was very cellular and showed increased granulopoiesis.

The results of the chromosome studies performed in February 1962, when the blood picture was substantially unchanged, are shown in Table II. When the patient was last seen in April 1962 there were no abnormal clinical findings other than those related to the periostitis. A more recent follow-up has not been possible.

Case 4

This patient, a woman aged 27 in 1962, was admitted to hospital in December 1962 with acute lower abdominal pain. Laparotomy revealed a haemoperitoneum due to bleeding from a ruptured cyst in each ovary; these were oversown. Haemostasis proved difficult throughout the operation and the post-operative period was complicated by a haematoma in the abdominal wall. A blood count on the day after the operation showed a severe leucocytosis: Hb 8.8 g./100 ml.; W.B.C. 110,000/c.mm. (neutrophils 82%, lymphocytes 6%, monocytes 2%, metamyelocytes 6%, neutrophil myelocytes 4%); 1 nucleated red cell/100 white cells; platelets 726,000/c.mm. The W.B.C. was still between 60,000 and 100,000/c.mm. when chromosome studies were carried out on two specimens of blood, one taken five days and the other 20 days post-operatively. The findings are given in Table II.

A satisfactory haematological remission was obtained by treatment with busulphan started on 25 December 1962. At no stage of the illness has there been any enlargement of the spleen, liver, or lymph nodes.

Case 5

A raised W.B.C. was noted when this patient, a man aged 61 in 1963, was admitted to hospital in March 1963 with incipient gangrene of the left foot. The findings were: Hb 12.3 g./100 ml.; W.B.C. 51,000/c.mm. (neutrophils 62%, lymphocytes 6%, monocytes 2%, eosinophils 2%, basophils 2%, metamyelocytes 7%, neutrophil myelocytes 15%, promyelocytes 2%, blasts 2%); 3 nucleated red cells/100 white cells; platelets 382,000/c.mm. when the initial chromosome studies were performed (Table II). Despite a left lumbar sympathectomy (15 March) the peripheral vascular disease progressed, necessitating a below-knee amputation of the left leg (22 March) from which recovery was uneventful.

X-ray therapy to the splenic region produced a satisfactory haematological remission, but the patient was readmitted in May with a right hemiplegia, and despite temporary improvement died on 31 May 1963, when he collapsed with severe abdominal pain. Necropsy revealed widespread atheroma with cerebral, splenic, and pulmonary infarction; small-bowel obstruction with perforation and a localized abscess and leukaemic infiltration of the spleen, liver, and bone-marrow.

Discussion

Not only is the presence of the Philadelphia chromosome in the leukaemic cells in most instances of chronic myeloid leukaemia well established, but it appears to be specific for this disorder as it has not been found in the blood or bone-marrow of patients suffering from other forms of leukaemia or primary bone-marrow disease (Baikie *et al.*, 1961; Hungerford, 1961; Hungerford and Nowell, 1962; Kemp *et al.*, 1963; Nowell and Hungerford, 1962; Sandberg *et al.*, 1962b). Before the exact relation between this disease and its chromosome abnormality can be understood, certain problems remain unresolved. Among these is the probability that in Ph¹+ cases not only the myeloid cells but also the erythroid and megakaryocyte series in the marrow are Ph¹+ (Tough *et al.*, 1963), and, secondly, the occurrence of the rare case of otherwise typical chronic myeloid leukaemia in which the Ph¹ chromosome cannot be detected in the blood or marrow cells despite exhaustive searching (Tough *et al.*, 1963). Little is known about the timing of the appearance of Ph¹+ cells in relation to the development of the disease, and it is with this problem in mind that the present series is reported.

The findings from Case 1 clearly illustrate that Ph¹+ cells are already present in the bone-marrow before there is any evidence of a leukaemic process in the peripheral blood and that the emergence of these Ph¹+ cells into the circulation coincides with the appearance of granulocyte precursors and the development of the typical blood picture. In each of the present cases Ph¹+ cells were observed in the marrow and/or

TABLE II.—*Cases 2–5. Chromosome Counts and Relevant Haematological Data*

Case	Specimen	Date	Total No. of Cells	Chromosome Number					Ph¹ %	Duration *in vitro* of cultures Assessed (Hours)	W.B.C./ c.mm.	N. %	L. %	M. %	E. %	B. %	Mm. %	My. %	Pro. %	Bl. %	L.A.P. Score
				<45	45	46	47	>47													
2	Blood*	12/11/62	41	7	4	30	—	—	95	42	48,700	71	9	1	3	—	—	13	3	—	26
3	„ †	26/2/62	35	—	—	35	—	—	75‡	42, 72	25,800	43	12	2	3	3	19	16	2	—	3
4	„ †	17/12/62	32	4	1	27	—	—	100	72, 90	81,000	59	9	3	4	2	13	9	1	—	31
	„ †	1/1/63	65	2	4	59	—	—	25‡	42, 66, 90	70,000	48	11	1	4	2	18	15	1	—	30
5	„ †	12/3/63	28	2	4	22	—	—	14	66	51,000	62	6	2	2	2	7	15	2	2	2
	„ †	22/3/63	10	2	—	8	—	—	57‡	72, 90	73,000	78	3	2	2	—	6	7	2	—	—
	Marrow (direct)	22/3/63	12	4	2	6	—	—	100	—											

* Culture without phytohaemagglutinin. †Culture with phytohaemagglutinin. ‡ Incidence of Ph¹+ cells fell during culture. For abbreviations, see Table I.

blood during the initial stages of the disease, when no clinical manifestation was apparent and when the haematological findings were equivocal.

The ultimate development of the typical leukaemic blood-picture in Cases 1 and 2 illustrates the inevitability of the progressive proliferation of these Ph^1+ cells and underlines their diagnostic importance when found in the blood or marrow of an individual without any other evidence of the disease. The value of cytogenetic studies in the diagnosis of either early or equivocal chronic myeloid leukaemia is greater than that of the assessment of L.A.P. activity; for although in three of the present cases the L.A.P. score was of the very low levels characteristic of chronic myeloid leukaemia, in two cases it was within the normal range. It is of interest that in these two cases the distribution of alkaline phosphatase activity was atypical, there being more cells with scores of 2 and even 3 than would be expected from normal blood with a similar total L.A.P. score. That this may reflect a reaction to leukaemic cells by *normal* myeloid cells in the earlier stages of the disease is suggested by the occurrence of one Ph^1- cell in the leucocyte cultures without phytohaemagglutinin in Case 2.

Sandberg *et al.* (1962b) have suggested that the occasional subjects with chronic myeloid leukaemia lacking the Ph^1 chromosome may be patients with a leukaemic picture complicating polycythaemia vera, while Wahrman *et al.* (1962) refer to the possibility that cases of chronic myeloid leukaemia following polycythaemia vera might represent a special leukaemic entity with its own chromosomal characterization. In Case 1 our findings lend support to neither of these possibilities, while the absence of anything like the manifold chromosome abnormalities described by Wahrman *et al.* (1962) supports the contention that these were the result of the extensive irradiation received by their patient (Court Brown *et al.*, 1962).

The hyperdiploid Ph^1+ cell lines observed in Case 1 during the development of the acute phase are thought to represent the blast-cell population which, incidentally, was noted to divide earlier *in vitro* (within 24 hours) than the myelocytes (approximately 48 hours) which had been circulating during the chronic phase. Although initially cells with a range of karyotypes were produced, all probably derived from the 46 Ph^1+ chronic myeloid cells, in the final stages of the acute phase one cell line became dominant. This sequence of events suggests the type of mechanism by which the established aneuploid cell lines reported in some patients with acute leukaemia (Baikie *et al.*, 1961; Ford, 1961; Sandberg *et al.*, 1961, 1962a; Tough *et al.*, 1961, 1963; Hungerford and Nowell, 1962), either arising *de novo* or following chronic myeloid leukaemia, have been selected. In this laboratory a comparable sequence of events has been observed during the early stages of the development of a stem-cell leukaemia. That the final cell-line in Case 1 should contain 49 chromosomes is of interest in view of other reported instances in which cells with the same chromosome number have predominated (Tough *et al.*, 1961; F. J. W. Lewis, personal communication, 1963). The persistence of the Ph^1 chromosome in the blast cells during the acute phase contrasts with the findings of Nowell and Hungerford (1961).

The consistent occurrence of aneuploidy coincided with treatment by alkylating agents and antimetabolites, but, although the possibility cannot be excluded that these chemicals may indirectly have influenced the chromosomal stability of the 46 Ph^1+ cells, it seems unlikely that the changes were the direct result of their action. Such changes have not been found in other patients receiving these drugs; the aneuploidy occurred only in the Ph^1+ cells, all the Ph^1- cells in cultures H and I possessing the normal male karyotype, and, furthermore, structural abnormalities reported to have been induced by alkylating agents (Conen and Lansky, 1961) such as dicentrics and fragments, were not seen in any of the aneuploid cells.

In the present state of our knowledge of the earliest stages of leukaemia it is possible that the control of chronic myeloid leukaemia would be more effective if treatment could be started before the disease process is haematologically fully manifest. Apart from providing additional and fundamental information about the leukaemic cell, chromosome study is currently the only means of achieving a definitive diagnosis during these early stages. Such cases might most profitably be sought by the investigation of selected groups of patients, such as those suffering from forms of primary bone-marrow diseases, known ultimately to be associated with a high incidence of chronic myeloid leukaemia, and patients in whom an increase in the number of circulating granulocytes cannot readily be explained.

Summary

The results of chromosome studies on five patients in the early stages of chronic myeloid leukaemia are reported. In each case Ph^1+ cells were found in the blood and/or bone-marrow before the disease was clinically apparent, and in one instance before there was any evidence of the leukaemia in the peripheral blood. The value of chromosome study in the diagnosis of this disorder is discussed with particular reference to the possibility that treatment might prove to be more effective if instituted before the disease is fully manifest.

One case was studied both during the development of the chronic phase and when a terminal acute myeloblastic transformation supervened. Initially, the latter was associated with the appearance of hyperdiploid Ph^1+ cells with as many as 52 chromosomes, although a 49 Ph^1+ cell line dominated the final stages. The possible significance of these findings is discussed.

We are grateful to Dr. T. R. E. Pilkington, Mr. R. Cox, Dr. A. L. Jacobs, Mr. G. Westbury, Dr. K. A. Newton, Professor H. Ellis, and Dr. J. G. Humble for permission to study these patients while under their care. Miss P. Carr and Miss E. Carman provided expert technical assistance. We also acknowledge the financial support of the British Empire Cancer Campaign and the Leukaemia Society Inc., so enabling this work to be carried out.

References

Baikie, A. G., Court Brown, W. M., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M. (1960). *Nature (Lond.)*, **188**, 1165.
—— Jacobs, P. A., McBride, J. A., and Tough, I. M. (1961). *Brit. med. J.*, **1**, 1564.
Conen, P. E., and Lansky, G. S. (1961). Ibid., **2**, 1055.
Court Brown, W. M., Jacobs, P. A., Tough, I. M., and Baikie, A. G. (1962). *Lancet*, **1**, 1242.
Fitzgerald, P. H., Adams, A., and Gunz, F. W. (1963). *Blood*, **21**, 183.
Ford, C. E. (1961). *Proc. 8th Congr. Europ. Soc. Haemat. Vienna*, **1**, 104.
—— Jacobs, P. A., and Lajtha, L. G. (1958). *Nature (Lond.)*, **181**, 1565.
Hayhoe, F. G. J., and Quaglino, D. (1958). *Brit. J. Haemat.*, **4**, 375.
Hungerford, D. A. (1961). *J. nat. Cancer Inst.*, **27**, 983.
—— and Nowell, P. C. (1962). Ibid., **29**, 545.
Kemp, N. H., Stafford, J. L., and Tanner, R. K. (1961). *Proc. 8th Congr. Europ. Soc. Haemat., Vienna*, **1**, 92.
—— —— —— (1963). Unpublished observations.
Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A. (1960). *Exp. Cell. Res.*, **20**, 613.
Nowell, P. C., and Hungerford, D. A. (1960a). *Science*, **132**, 1497.
—— —— (1960b). *J. nat. Cancer Inst.*, **25**, 85.
—— —— (1961). Ibid., **27**, 1013.
—— —— (1962). Ibid., **29**, 911.
Sandberg, A. A., Ishihara, T., Crosswhite, L. H., Hauschka, T. S. (1962a). *Cancer Res.*, **22**, 748.
—— —— —— —— (1962b). *Blood*, **20**, 393.
—— —— Miwa, T., and Hauschka, T. S. (1961). *Cancer Res.*, **21**, 678.
Tjio, J. H., and Whang, J. (1962). *Stain Technol.*, **37**, 17.
Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A. (1961). *Lancet*, **1**, 411.
—— —— —— —— —— —— and Williams, J. A. (1962). Ibid., **2**, 115.
—— Jacobs, P. A., Court Brown, W. M., Baikie, A. G., Williamson, E. R. D. (1963). Ibid., **1**, 844.
Wahrman, J., Schaap, T., and Robinson, E. (1962). Ibid., **1**, 1098.